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PATENT

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Applicant: Frank Karlsen

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Examiner: Jehanne E. Souaya

For: METHOD FOR IDENTIFICATION OF THE INDICATORS OF CONTAMINATION IN LIQUID SAMPLES

Box Fee Amendment
Commissioner for Patents
Washington, D.C. 20231

Amendment and Response

In response to the Office action of March 28, 2002, please amend the above-identified application as follows.

**Due to the indefinite suspension of U.S. Express Mail services and pursuant to the PTO instructions of 11/19/2001, the enclosed documents are being deposited for U.S. Express Mail in an envelope addressed to: BOX FEE AMENDMENT COMMISSIONER FOR PATENTS, 2900 CRYSTAL DRIVE, ARLINGTON, VA 22202-3513. IT IS OUR UNDERSTANDING THE PTO WILL BE RESPONSIBLE FOR FORWARDING ALL OF THE ENCLOSED DOCS. TO THE PTO OFFICE LOCATED IN WASHINGTON D.C.

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In the Specification:

Please replace the paragraph beginning at page 10, line 16, with the following rewritten paragraph:

--The present invention provides a method for detecting water-borne microorganisms which serve as an indicator of the probable presence of pathogens, primarily of fecal origin. In a preferred embodiment, the method comprises the following steps: (1) providing a liquid or liquified sample (which may include treating the sample with a culture media to enrich microorganisms present in the sample); (2) recovering microorganisms from the treated sample; (3) lysing the recovered microorganisms to release substantially undegraded DNA therefrom; (4) providing specific primers which will hybridize to separated target strands of a target nucleotide sequence from the target gene of the microorganism of interest and amplify the target nucleotide sequence but not sequences found in other organisms; (5) mixing the primers with the recovered DNA; (6) amplifying the specific target nucleotide sequence with polymerase thereby extending the primers to make fully double-stranded replicas of the target DNA sequence; (7) detecting the presence of the amplicon (amplified target DNA sequence) by binding the amplicon to a microtiter plate and staining with a stain such as PICOGREEN, which selectively stains double stranded DNA; and (8) making a determination about the presence or absence of the microorganism in the original liquid or liquified sample based on

presence or absence of the amplicon. Examples of the primers which may be used, and which are discussed in more detail below, are shown in Tables I, and II, III and IV.--

Please replace the paragraph beginning at page 32, line 19, with the following rewritten paragraph:

--In a preferred version, the detection reagent is the substrate PICOGREEN. The use of PICOGREEN as a stain for double stranded DNA (dsDNA) is described in U.S. Pat. No. 5,824,557 entitled, "Method for Detecting and Quantitating Nucleic Acid Impurities in Biochemical Preparations", issued Oct. 20, 1998 to T. J. Burke *et al* and assigned to PanVera Corporation, which is hereby expressly incorporated herein by reference. PICOGREEN is an ultra sensitive fluorescent nucleic acid stain which is ideal for quantitating dsDNA in the microtiter plate format. The ability of this reagent to detect very small amounts of dsDNA in the presence of contaminating RNA, single stranded DNA (ssDNA) or proteins, combined with its wide linear response, provides significant advantages over other methods.--

Please replace the paragraph beginning at page 33, line 8, with the following rewritten paragraph:

--Free PICOGREEN dye is essentially nonfluorescent and exhibits a greater than 1,000-fold fluorescence enhancement upon

binding to dsDNA. PICOGREEN staining is highly selective for dsDNA over RNA, ssDNA and oligonucleotides, and is not compromised by the presence of proteins, nucleotides and other sample contaminants in the reaction.--

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Please replace the paragraph beginning at page 33, line 14, with the following rewritten paragraph:

--However, it will be understood by a person of ordinary skill in the art that although PICOGREEN is a preferred stain, other detection reagents, including other dsDNA stains well known to persons of ordinary skill in the art, may be used in the present invention.--

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Please replace the paragraph beginning at page 33, line 19, with the following rewritten paragraph:

--The detection reagent controls which contain only PICOGREEN(or other detection reagent) and each of the primer sets are used to determine a baseline fluorescence for determining presence or absence of *E. coli* and/or *Enterococcus faecalis/faecium*.--

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Please replace the paragraph beginning at page 34, line 16, with the following rewritten paragraph:

--The detection method as described in detail herein is

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designed to detect the presence or absence of the indicator organism. However, a semiquantitative result can also be obtained using a similar method. In this method, to a series of wells is added increasing known amounts of *E. coli* or *Enterococcus* so that a standard curve can be constructed. The result obtained from the DNA sample collected from a liquid or liquified sample can be directly compared to the standard curve to determine the approximate amount of indicator organism present in the liquid or liquified sample. The use of PICOGREEN or other detection reagents will allow for quantitation of the dsDNA present, and the assay will display a linear correlation between dsDNA concentration and fluorescence, allowing a detection range extending from about 25 pg/ml to about 1 µg/ml dsDNA using a single PICOGREEN dye concentration.--

B6

Please replace the paragraph beginning at page 35, line 12, with the following rewritten paragraph:

--In one embodiment, a desired kit for use in a method for detecting *Escherichia coli* in a liquid or liquified sample comprises a primer set for amplification of a sequence in the *lamB* gene. The primer set is selected from the primer sets described in Table V or elsewhere herein such as primer sequences of up to 40 bp comprising SEQ ID NO:1 and SEQ ID NO:14. One of the two primer sequences of the primer set provided in the kit will be

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biotinylated. The kit may also include a detection reagent, such as PICOGREEN, for detection of an amplified sequence in the *lamB* gene, and a detection well having streptavidin coated thereon wherein the amplified sequence is detected by the detection reagent.--

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Please replace the paragraph beginning at page 36, line 1, with the following rewritten paragraph:

--In another embodiment, a desired kit for use in a method for detecting *Enterococcus faecalis* and/or *Enterococcus faecium* in a liquid or liquified sample comprises a primer set for amplification of a sequence in the transposase gene Tn1546. The primer set is selected from the primer sets described in Table VI or elsewhere herein such as primer sequences of up to 40 bp comprising SEQ ID NO:27 and SEQ ID NO:32. One of the two primer sequences provided in the kit will be biotinylated. The kit may also include a detection reagent, such as PICOGREEN, for detection of an amplified sequence in the transposase gene Tn1546, and a detection well having streptavidin coated thereon wherein the amplified sequence is detected by the detection reagent. As noted above, kits may comprise primer sets for both *E. coli* and *Enterococcus faecalis/faecium* and may further comprise more than one primer set appropriate for each species of bacteria.--

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In the Claims:

Please cancel claims 7 and 15 without prejudice.

Please amend claims 1-3, 6, 8, 13-14, and 16-18, which are submitted herewith in clean, replacement form as follows. Also, submitted herein below following the Remarks section are redlined, marked up versions of claims 1-3, 6, 8, 13-14, and 16-18 which illustrate the amendments made to such claims.

Clean Version of Replacement Claims:

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1. (Twice Amended) An oligonucleotide primer having up to 40 bases and comprising the sequence SEQ ID NO:1; or SEQ ID NO:14 wherein the primer is specific for the detection of E. coli.

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2. (Once Amended) An oligonucleotide primer having 23-40 bases and comprising SEQ ID NO:2; or SEQ ID NO:3 wherein the primer is specific for the detection of E. coli.

3. (Once Amended) An oligonucleotide primer having 23-40 bases and comprising SEQ ID NO:15; or SEQ ID NO:16 wherein the primer is specific for the detection of E. coli.

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6. (Once Amended) A method of specifically detecting E. coli in a liquid or liquified sample by polymerase chain reaction, comprising:

providing a liquid or liquified sample;

recovering bacteria from the liquid or liquified sample;

lysing the bacteria to provide a DNA sample;

B10 treating the DNA sample under PCR conditions with a primer set specific for E. coli for forming an amplified DNA wherein the primer set comprises SEQ ID NO:1 and SEQ ID NO:14; and

detecting the presence of amplified DNA as an indication of the presence of E. coli in the liquid or liquified sample.

B11 8. (Once Amended) The method of claim 6 wherein in the step of detecting the presence of amplified DNA, the presence of *Escherichia coli* is indicated when a signal is obtained which exceeds a predetermined threshold.

13. (Twice Amended) A method of specifically detecting E. coli in a liquid or liquified sample by polymerase chain reaction, comprising:

providing a liquid or liquified sample;

recovering bacteria from the liquid or liquified sample;

lysing the bacteria to provide a DNA sample;

selecting a target gene of E. coli and selecting an E. coli-specific target DNA sequence in the target gene;

incubating the DNA sample under amplification conditions with a DNA polymerase and a primer pair specific for E. coli for amplifying the target DNA sequence; and

detecting the presence of amplified DNA as a specific indication of the presence of E. coli carrying the selected E. coli-specific target DNA sequence, wherein the target gene is the *lamB* gene of *Escherichia coli*.

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14. (Once Amended) A kit for use in specifically detecting *Escherichia coli* in a liquid or liquified sample, the kit comprising a primer pair having a first primer comprising SEQ ID NO: 1 and a second primer comprising SEQ ID NO: 14.

16. (Once Amended) The kit of claim 14 further comprising a detection agent for detection of amplified DNA produced using the primer pair under amplification conditions.

B18 17. (Once Amended) The kit of claim 16 wherein the detection reagent is a dsDNA stain.

18. (Once Amended) The kit of claim 16 further comprising a detection well having streptavidin coated thereon wherein the amplified DNA sequence is detected by the detection reagent.

Please add the following new claims: 26-39.

26. (New) A method of specifically detecting E. coli in a liquid or liquified sample by polymerase chain reaction, comprising:

providing a liquid or liquified sample;

recovering bacteria from the liquid or liquified sample;

lysing the bacteria to provide a DNA sample;

treating the DNA sample under PCR conditions with a primer set specific for E. coli for forming an amplified DNA wherein the primer set comprises SEQ ID NO:2 and SEQ ID NO:15; and

detecting the presence of amplified DNA as an indication of the presence of E. coli in the liquid or liquified sample.

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27. (New) The method of claim 26 wherein in the step of detecting the presence of amplified DNA, the presence of *Escherichia coli* is indicated when a signal is obtained which exceeds a predetermined threshold.

28. (New) A method of specifically detecting E. coli in a liquid or liquified sample by polymerase chain reaction, comprising:

providing a liquid or liquified sample;

recovering bacteria from the liquid or liquified sample;

lysing the bacteria to provide a DNA sample;

treating the DNA sample under PCR conditions with a primer set specific for E. coli forming an amplified DNA wherein the primer set comprises SEQ ID NO:3 and SEQ ID NO:16; and

detecting the presence of amplified DNA as an indication of the presence of E. coli in the liquid or liquified sample.

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29. (New) The method of claim 28 wherein in the step of detecting the presence of amplified DNA, the presence of *Escherichia coli* is indicated when a signal is obtained which exceeds a predetermined threshold.

30. (New) A kit for use in detecting *Escherichia coli* in a liquid or liquified sample, the kit comprising a primer pair having a first primer comprising SEQ ID NO: 2, and a second primer comprising SEQ ID NO: 15.

31. (New) The kit of claim 30 further comprising a detection agent for detection of the amplified DNA produced using the primer pair under amplification conditions.

32. (New) The kit of claim 31 wherein the detection reagent is a dsDNA stain.

33. (New) The kit of claim 31 further comprising a detection well having streptavidin coated thereon wherein the amplified DNA sequence is detected by the detection reagent.

B14 34. (New) The kit of claim 30 wherein one of the first primer and the second primer is biotinylated.

35. (New) A kit for use in detecting *Escherichia coli* in a liquid or liquified sample, the kit comprising a primer pair having a first primer comprising SEQ ID NO: 3 and a second primer comprising SEQ ID NO: 16.

36. (New) The kit of claim 35 further comprising a detection agent for detection of the amplified DNA produced using the primer pair under amplification conditions.

37. (New) The kit of claim 36 wherein the detection reagent

is a dsDNA stain.

38. (New) The kit of claim 36 further comprising a detection well having streptavidin coated thereon wherein the amplified DNA sequence is detected by the detection reagent.

39. (New) The kit of claim 35 wherein one of the first primer and the second primer is biotinylated.

Remarks

This is intended to be a complete response to the official action mailed March 28, 2002 in which claims 1-3, 6-8, and 13-19 were rejected. Applicant has amended claims 1-3, 6, 8, 13, 14 and 16-18 herein, has cancelled claims 7 and 15 without prejudice, and has added new claims 26-39. Now claims 26-39 are similar to amended claims 6, 8, 6, 8, 14, 16-19, 14, and 16-19, respectively.

Priority

The claims have been amended so that claims 1, 6, and 14 are directed to primers with SEQ ID NO: 1 and 14, claims 2, 26 and 30 are directed to primers with SEQ ID NO: 2 and 15, and claims 3, 28 and 32 are directed to primers with SEQ ID NO: 3 and 16.

Applicant respectfully traverses examiner's assertion that SEQ ID NO: 2, 3, 15, and 16 are not due the benefit of the priority date (August 13, 1999) of the provisional application 60/149, 365. On page 23, lines 5-11, of the provisional application it is stated that the invention comprises primer sequences which comprise "a substantial part" of SEQ ID NO: 1 and SEQ ID NO: 2 [Note: SEQ ID NO: 2 in the provisional application is SEQ ID NO: 14 in the present application].

It is readily visible from Table I that SEQ ID: 2 and SEQ ID: 3 each comprise "a substantial part" of SEQ ID NO: 1. Further it can be seen from Table II that SEQ ID NO: 15 and SEQ ID NO: 16 each

comprise "a substantial part" of SEQ ID NO: 14.

Thus, applicant respectfully submits each of SEQ ID NO: 1, 2, 3, 14, 15, and 16 are taught and enabled by the parent provisional application and thus deserve the benefit of the (August 13, 1999) filing date thereof.

Trademarks

The specification has been updated to refer to trademarked names in capital letters.

Sequence Listing

A corrected substitute sequence listing has been supplied herewith, to correct the errors identified in the official action.

Rejections Under § 112 ¶ 2

Claim 6-8 and 14-19 have been rejected under § 112 ¶ 2 as being indefinite.

Applicant has amended the claims to improve their clarity in accordance with the examiner's suggestions. In view of the amendments to the claims, applicant respectfully submits the claims are now clear and definite and respectfully requests reconsideration and withdrawal of the rejection of the claims under 35 U.S.C. § 112 ¶ 2.

Rejections Under § 102(b)

Claim 13 stands rejected under 35 U.S.C. 102(b) as being anticipated by Atlas et al. (U.S. Patent 5,298,392; 3/29/1994).

Atlas et al. teach a method of detecting E. coli in a sample wherein the target is the E. coli LamB gene. While Atlas et al. do teach primer sequences which target the LamB gene, the primer sequences taught by Atlas et al are not specific for E. coli. Atlas et al. teach that even at higher annealing temperatures, Shigella spp., are detected using the same LamB primer sets which are used to detect E. coli. (Col. 17, lines 33-66). Atlas et al. thus do not teach LamB primers which are specific for E. coli.

Applicant, has amended claim 13 to be directed to a method of detecting E. coli which is specific for E. coli., wherein a LamB primer set which is specific for E. coli. is used. As noted above, Atlas et al. do not teach a method which is specific for E. coli. Atlas et al. therefore does not teach each and every element of the invention as claimed in amended claim 13. In view of the above, applicant respectfully requests reconsideration and withdrawal of the rejection of claim 13 under 35 U.S.C. §102(b).

First Rejections Under § 103(a)

Claims 1-3, 6-8, and 14-16 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Atlas et al. (U.S. Patent 5,298,392; 3/29/1994) in view of Clement et al. (Cell, vol. 27, pp 507-514, Dec. 1981).

Applicant has amended claims 1-3, 6, 8, 14, and 16-18 to be directed to methods and kits which use and contain primers which are specific for E. coli. Neither of the references teach methods or primers which are specific for E. coli. While Clement et al. may teach a large DNA sequence which contains the sequences used as primers in the present inventions, or sequences complementary thereto, there is no evidence provided in Atlas et al. or Clement et al., that these sequences are capable of specifically detecting E. coli. Contrary to examiner's assertion on page 9, lines 1-4, the sequences claimed herein are not functionally equivalent to just any sequence derived from Figure 1 of Clement et al. because the present sequences are specific for E. coli., while Atlas et al. teach only LamB sequences which are not specific for E. coli.

In view of the above, applicant respectfully submits that the present claims are not obvious over Atlas et al., in view of Clement et al. and respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. § 103(a).

Second Rejection Under § 103(a)

Claim 17 stands rejected under 35 U.S.C. 103(a) as being unpatentable over Atlas et al. in view of Clement et al. as applied to claims 1-3, 6-8, and 14-15 above, and further in view of the Molecular Probes Catalog (6th edition, 1996, pp. 161-162; Handbook of Fluorescent Probes and Research Chemicals).

The molecular Probes Catalog does not remedy the deficiencies of Atlas et al. and Clement et al. In view of the response provided to the first rejection under 103(a), applicant respectfully requests reconsideration and withdrawal of the rejection.

Third Rejection Under § 103(a)

Claims 18-19 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Atlas in view of Clement as applied to claims 1-3, 6-8, and 14-15 above, further in view of Challberg et al. (WO 93/10623, May 27, 1993), and further in view of Biosystems Reporter (vol. II, 1996, pages 1-3).

Applicant respectfully traverses the rejection for the same reasons given above in the response to the first rejection under § 103(a).

The Challberg et al. and Biosystems Reporter references do not remedy the deficiencies of the Atlas et al. and Clement et al. references. In view of the above, applicant respectfully requests

reconsideration and withdrawal of the rejection under 35 U.S.C. § 103(a).

Conclusion

In view of the above, applicant respectfully submits the claims are now in a condition for allowance and requests issuance of a Notice of Allowance thereof.

Marked Up Version of the Claims and Abstract

Attached hereto is a marked up version of the changes made to the claims and abstract by the current amendment. The attached page is captioned "Version With Markings To Show Changes Made".

Respectfully submitted,

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